Use of Biomarker Sequences for the Identification and Phylogenic Analysis of Filamentous Fungi Isolated from Extreme Environments

Tamas Torok, Nelli Zhdanova, Mykola Kuchuk, Glen Dahlbacka, Gary Andersen, Veronica Amaku, and Jennie Hunter-Cevera
Contact: Tamas Torok, 510/486-5808, ttorok@lbl.gov

RESEARCH OBJECTIVES

Fungi play complex and diverse roles in ecosystems. Their importance to biotechnology and bioprospecting is enhanced by the vast diversity of extant fungi. The search for novel capabilities and useful new genetic traits most often targets microorganisms that live under extreme environmental conditions, such as high temperature, extreme pH, salinity, and radiation. Scientists at Berkeley Lab's Center for Environmental Biotechnology have been bioprospecting for many years at contaminated sites and closed military bases, in deserts and forests in the USA, in Lake Baikal sediments and at geothermal and hydrothermal sites on the Kamchatka peninsula in Russia, and at the failed nuclear power plant and within the surrounding 30 km "Exclusion Zone" in Chernobyl, Ukraine. Their work focuses on filamentous fungi that often possess the ability to produce unique secondary metabolites with potential commercial value. These natural products are advantageous to the organism in their respective environment as chemical defense against predators, pathogens, or competitors. Identification of these microorganisms provides a better understanding of their ecological function. The overall goal of this project is the taxonomic characterization of thousands of fungal cultures in a way that is amenable to a much wider range of laboratories.

APPROACH

Procedures traditionally used for fungal identification rely on colony- and cell morphology and other distinctive biochemical reactions. Recently, molecular-level protocols have been increasingly used for fungal identification. Here, we applied a polymerase chain reaction (PCR) combined with amplicon sequencing and comparative sequence analysis of biomarker genes. DNA sequencing was done at the University of California-Berkeley DNA Sequencing Facility. Raw sequences were edited and aligned using online multiple sequence aligner subroutines. Aligned sequences were further analyzed for consensus and finally queried against the National Center for Biotechnology Information database for species determination (Figure 1).

ACCOMPLISHMENTS

The main objective of this study was to test an alternative method of fungal identification. Though the number of strains/species/genera included in this study was biased by the extreme environmental habitats, most of over 100 species tested so far were identified correctly, in agreement between classical and molecular-level identification. More definite results will be available when the sequence-based identification of the over 2,000 filamentous fungi isolated from extreme environmental samples is completed.

SIGNIFICANCE OF FINDINGS

Taxonomic identification and detailed characterization of extremophillic microorganisms provides a greater understanding of their diversity, unique metabolism, and ecological function. Here, fungal organisms were characterized successfully by comparing classical techniques and sequence-based phylogenetic differentiation. Because we consider filamentous fungi major sources of novel biotechnology and biomedical applications, a future goal is to design an Affymetrix-type microarray to aid in better understanding of fungal phylogenetic relatedness and diversity.

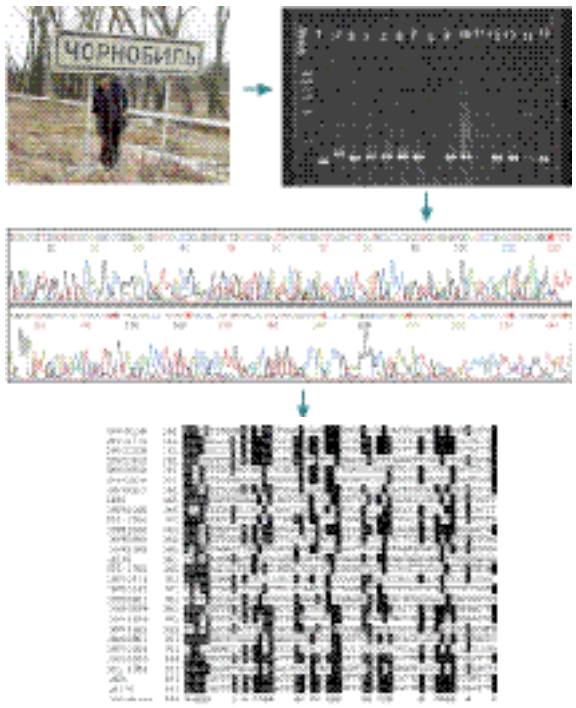

Figure 1. Flow diagram of the sequence-based identification (from environmental sample to the consensus of aligned sequences)

RELATED PUBLICATIONS

Torok, T., N. Zhdanova, M. Kuchuk, G. Dahlbacka, Amaku, G. Andersen, and J. Hunter-Cevera, Characterization of filamentous fungi isolated from extreme environments. Proceedings of the Fifth International Conference on Extremophiles ("Extremophiles 2004"), p. 86, Cambridge, Maryland, September 19–23, 2004.

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